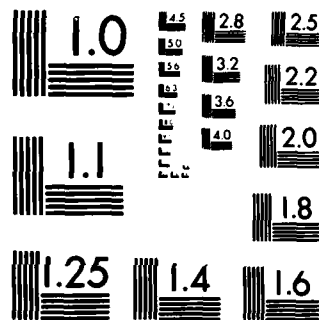


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LOCAL ANESTHETIC MICROENCAPSULATION
Annual Report

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June 14, 1982
(For period September 30, 1981 through May 31, 1982)

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Woburn, Massachusetts 01801

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Because of the natural molecular weight distribution of poly-L(-)lactide polymerization, batches can be blended with viscosities as disparate as R.S.V. = 1.2 ± 0.6 dl/g. Microencapsulation of lidocaine (base) yielded 212-300 micron microcapsules with 50% in vitro drug release in 6 hours; 150-212 micron microcapsules released 50% in 2 hours. Etidocaine-HCl and bupivacaine-HCl were microencapsulated in a more efficient manner. A wide variety of drug release profiles was obtained. Microcapsules of 300-425 microns in size containing 60% bupivacaine-HCl had 50% in vitro drug release in 12 hours.			

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EDAX of bupivacaine HCl microcapsules indicate about 25% drug on the microcapsule surface. One year storage of lidocaine HCl and etidocaine HCl microcapsules do not show significant differences of drug assay or in vitro release.

Acute systemic toxicity of free and encapsulated drugs were compared by intraperitoneal injection in mice. Lethal and convulsive doses (LD50, CD50) were between 4.0 and 6.7 times as great with anesthetics in the encapsulated form.

Cutaneous anesthesia was measured with an electromyograph and a tactile stimulator. Intradermal studies with anesthetic solutions showed that the guinea pig was a better model than the rabbit. Subcutaneous studies with microcapsule suspensions and anesthetic solutions are in progress.

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FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Citation of commercial organizations and trade names in this report does not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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I. SUMMARY

Because of the natural molecular weight distribution of poly-L(-)lactide polymerization, batches can be blended with viscosities as disparate as $R.S.V. = 1.2 \pm 0.6$ dl/g. Microencapsulation of lidocaine (base) yielded 212-300 micron microcapsules with 50% in vitro drug release in 6 hours; 150-212 micron microcapsules released 50% in 2 hours. Etidocaine·HCl and bupivacaine·HCl were microencapsulated in a more efficient manner. A wide variety of drug release profiles was obtained. Microcapsules of 300-425 microns in size containing 60% bupivacaine·HCl had 50% in vitro drug release in 12 hours. EDAX of bupivacaine·HCl microcapsules indicate about 25% drug on the microcapsule surface. One year storage of lidocaine·HCl and etidocaine·HCl microcapsules do not show significant differences of drug assay or in vitro release.

Acute systemic toxicity of free and encapsulated drugs were compared by intraperitoneal injection in mice. Lethal and convulsive doses (LD50, CD50) were between 4.0 and 6.7 times as great with anesthetics in the encapsulated form.

Cutaneous anesthesia was measured with an electromyograph and a tactile stimulator. Intradermal studies with anesthetic solutions showed that the guinea pig was a better model than the rabbit. Subcutaneous studies with microcapsule suspensions and anesthetic solutions are in progress.

II. ACCOMPLISHMENTS

A. In Vitro Studies

1. Polymer Availability

In the proposal we indicated that the use of poly-L(-)lactide of R.S.V. = 1.2 dl/g from batches of R.S.V. between 1.0 and 1.5 dl/g was unnecessarily restrictive. Since the polymerization process produces a wide range of molecular weights, a less restrictive R.S.V. range is warranted.

At the start of the contract period we had approximately 450 grams of the polymer blend (R.S.V. = 1.19 dl/g). This material was prepared for Contract Nos. DAMD17-80-C-0093 and DAMD17-80-C-0110 and can be used on this contract and Contract No. DAMD17-81-C-1204. Based on the broad molecular weight distribution of the previously prepared polymer, we now believe that a broad range of viscosities (R.S.V.) can be blended, without affecting the final molecular weight distribution. This broad molecular weight distribution was also found to be natural for the process. The theoretical polydispersity of a completed condensation polymerization reaction is 2.0 (Flory, 1953). The polydispersity (MW_w/MW_n) of our polymer is 2.14.

Polymer 5-6-27 of R.S.V. \sim 0.72 dl/g and Polymer 5-6-25 of R.S.V. \sim 2.23 dl/g were, therefore, precipitated with isopropanol:methylene chloride (3:1). These polymers were dried, ground, and stored in the freezer. At this time the viscosities were R.S.V. = 2.04 dl/g for Polymer 5-6-25 and R.S.V. = 0.73 dl/g for Polymer 5-6-27. Weights are 390 grams and 375 grams, respectively.

To prove that these batches could be blended, a sample of 0.73 dl/g polymer (5-6-27) and 2.04 dl/g polymer (5-6-25) were analyzed by gel permeation chromatography. Also a combination of 33% of the low and 67% of the high molecular weight polymer was analysed. The results are summarized in Table 1 and compared with the initial polymer analysis of Contract No. DAMD 17-80-C-0110. At equal concentrations of the two polymers (0.73 and 2.04 dl/g) the chromatogram overlap is 48% (Figure 1). The combination chromatogram does not show a bimodal distribution but does show a tailing at high molecular weights.

Thus we can probably blend this wide a viscosity range without affecting the polymer coating and drug release properties of the polymer blend. When needed, these polymers will be blended (dissolved in CH_2Cl_2) in a 1:2 ratio to give an R.S.V. of ~ 1.20 dl/g. Several additional polymer batches are presently available in the 0.6-0.8 dl/g range.

2. Microencapsulation of Anesthetics

Initial encapsulations of lidocaine, etidocaine, and bupivacaine hydrochlorides were performed on the previous contract. Based on these experiments, the goals of the present encapsulations were to:

- 1) prepare bupivacaine and etidocaine hydrochloride microcapsules to 30% polymer, coating in a more time-efficient manner,
- 2) using a less soluble form of lidocaine, prepare microcapsules which will release the drug more slowly.

Table 1
EFFECT OF VISCOSITY (R.S.V.) ON POLYMER BLENDS

<u>R.S.V.</u>	<u>Polymer</u>	<u>MW</u>	<u>MWn</u>	<u>MWw/MWn</u>
0.73	5-6-27	29,880	17,590	1.70
2.04	5-6-25	87,840	52,020	1.69
1.20*	Blend of Above	57,420	22,860	2.51
1.19	Previous Contract Blend	45,700	21,500	2.14

* calculated R.S.V.

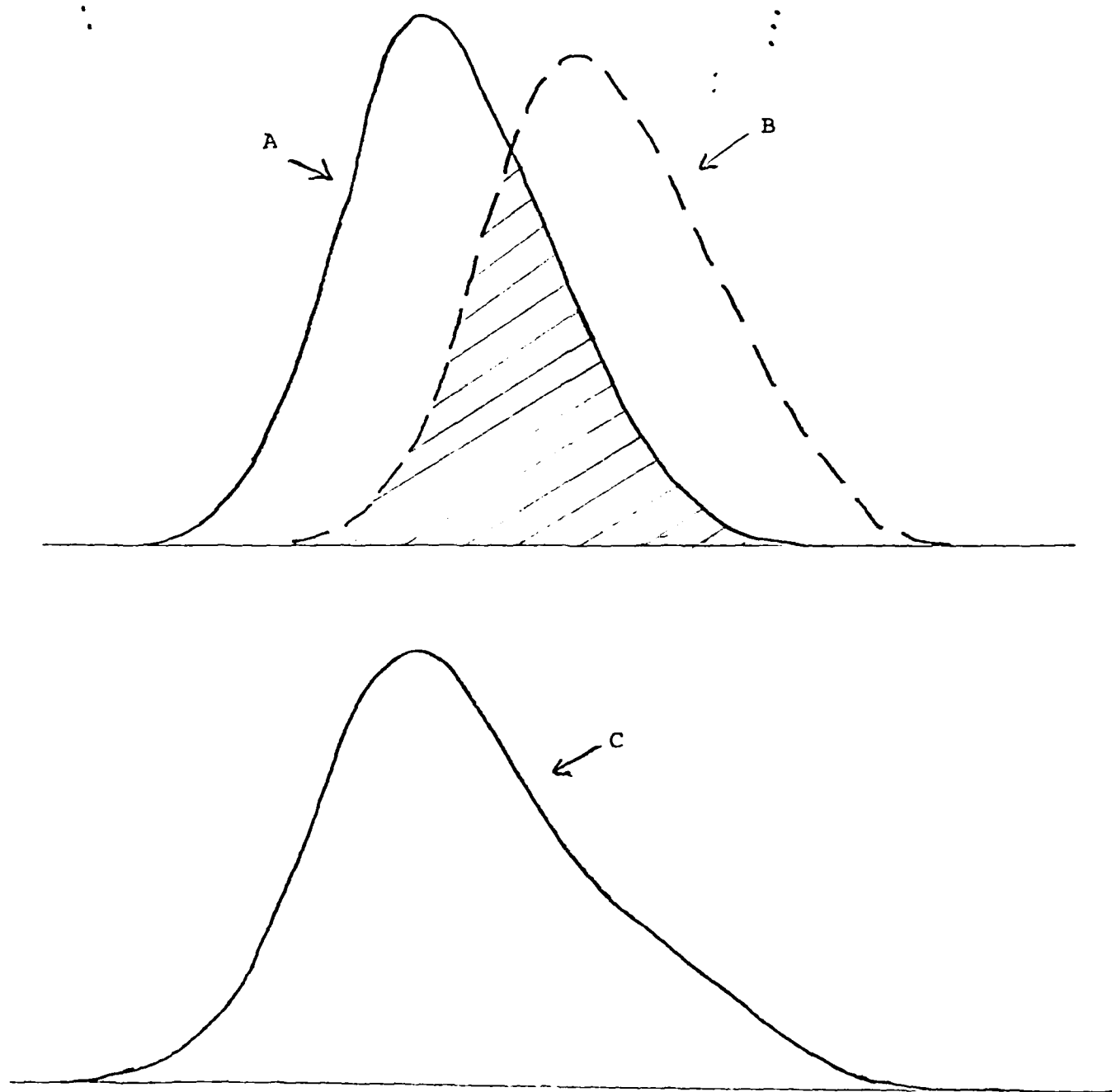


Figure 1 Gel Permeation Chromatograms of
A. 5-6-27, R.S.V. = 0.73 Pd = 1.70
B. 5-6-25, R.S.V. = 2.04 Pd = 1.69
C. 2 of 5-6-27 and 1 of 5-6-25
R.S.V. = 1.2 Pd = 2.51

a. Lidocaine Microencapsulation

A less soluble form of lidocaine than the hydrochloride should give slower release for the same microcapsule at the same drug loading. Thus sulfate and phosphate salts were prepared, in addition to use of the base form of lidocaine.

1) Lidocaine Salts

Lidocaine salts can be prepared by precipitation of the less soluble salt in water or by addition of the appropriate acid to the base form of the drug. Best results were obtained by dissolving the lidocaine (base) in ethanol and adding concentrated sulfuric or phosphoric acid solutions (aqueous). The precipitates were then washed with ethanol. Lidocaine sulfate has a melting point of $\sim 215^{\circ}\text{C}$ and is slightly soluble in water. Lidocaine phosphate has a melting point of $\sim 180^{\circ}\text{C}$, but it is very soluble in water. Thus the sulfate would be the salt of choice for encapsulation.

2) Lidocaine (Base) Recrystallization

Lidocaine (base), U.S.P., can be obtained from Sterling Organics, Inc. Since lidocaine (base) is extremely soluble in methylene chloride we were also concerned that the coating process might agglomerate the bed. Thus we were prepared to use the sulfate.

3) Lidocaine (Base) Encapsulation

Previous Wurster coatings were started at 20 ml/min. to agglomerate the drug particles. At 10% polymer coating, the coating rate had been reduced to 3 ml/min. However, in this run, severe agglomeration occurred at 10 ml/min., and the bed collapsed. The oversized material was ground through a Braun mill and placed back in the Wurster unit. Coating continued at 5 ml/min. and was stopped at 30% polymer. This required only two days of coating time. For comparison, the coating of lidocaine·HCl to 50% polymer had taken five days.

The summary of the results of the coating process is shown in Table 2. The microcapsule size distribution is shown in Table 3. The microcapsules are larger than had been expected, and there are considerable fines (brushed sample) which have a high drug content.

b. Etidocaine Microencapsulation

Etidocaine hydrochloride was also microencapsulated using the standard polymer (R.S.V. = 1.19 dl/g). The same materials were used as in the microencapsulation of the previous contract. However, the method of microencapsulation was similar to that described for lidocaine (base) above.

TABLE 2

PROCESSING SUMMARY OF LIDOCAINE (BASE) MICROENCAPSULATION

(Run 11-1)

Process Polymer %	Starting Weights		Final Weight	Losses			
	Starting Sample g	Polymer Added g		MC (as drug) g (g)	Wurster Holdup g	Oversize Removed g (500µm)	Sieve + Bag g
							Samples Removed g
0-13	300	33	186 (165)		148	ground	10
13-20	176	15	198 (165)		-7	55	30
20-30	112	16	122 (158)**		6 -84*	-	122 84*
TOTALS		64			63 -	55	246
	A	B	C		D	E	F
							G

Material Balance In: 300g drug + 64 g polymer = 364 g

Out: 246g samples + 163 Wurster + 55 oversize = 364 g

** Yield at 30% is 53% (not considering 40 g of samples) based on drug

** Yield at 30% is 68% based on samples/input material

$$D_n = A_n + B_n - C_n \quad A_{n+1} = C_n - (E_n + F_n + G_n)$$

* Brushed down of Wurster unit yields 84 grams

** Includes brushed-down material

TABLE 3
LIDOCAINE (BASE) MICROCAPSULE SIZE DISTRIBUTION
(Values are % of weight in each sieve fraction)

Microcapsules Size Range (μ m)	% Drug in Microcapsules		
	90%	80%	70%*
> 600	10	0	3
425-600	18	20	22
300-425	17	24	28
212-300	16	20	23
150-212	11	9.2	7.9
106-150	9.1	7.3	4.7
75-106	10.4	8.7	5.4
38-75	8.5	8.8	5.1
< 38	0.9	1.1	0.5

* Does not include brush-down material

Etidocaine·HCl fluidizes more readily than does lidocaine (base). The agglomerating phase in which the 3% polymer solution is introduced at 20 ml/min. (0-11% coating) caused only a slight agglomeration of particles. Particles larger than 250 microns (51 grams) were removed from the bed (see processing summary, Table 4). The coating was then continued at 10 ml/min.; but even this coating rate did not significantly increase the mean particle size. The entire coating time to reach the 30% coated sample was 3.4 hours. Almost 20 hours of coating was required for the previous etidocaine microencapsulations, with 10 hours being required to reach 30% polymer.

The size distribution of the microcapsules is shown in Table 5. This distribution is more similar to the previous etidocaine run than the lidocaine (base) run. However, some large particles are available for testing.

c. Bupivacaine Microencapsulation

Bupivacaine hydrochloride from Chem Biochem Research, Inc., Salt Lake City, Utah, was encapsulated in the same manner as described above. The material was intermediate in flowability between the sticky lidocaine (base) and the

TABLE 4

PROCESSING SUMMARY OF ETIDOCAINE·HCl MICROENCAPSULATION
(Run 11-2)

Process Polymer %	Starting Weights		Final Weight	Losses				
	Starting Sample g	Polymer Added g		MC (as drug) g (g)	Wurster Holdup g	Oversize Removed g (500 μ m)	Sieve + Bag g	Samples Removed g
0-11	300	36.5	203	(181)	133.5	$\left\{ \begin{array}{l} 44 \\ 51(>250) \end{array} \right\}$	4	11
11-20	137(<250)	15.5	176	(141)	-23.5	6	0	11
20-30	159	25.5	195 33*	(136) (23)	-10.5	-	-	$\left\{ \begin{array}{l} 195 \\ 33* \end{array} \right\}$
TOTALS		77.5			66.5	57	4	250**
	A	B	C		D	E	F	G

Material Balance In: 300 g drug + 77.5 g polymer = 377.5 g

Out: 250g samples + 57 g oversize + 66.5 g Wurster holdup + 4 g other losses = 377.5 g

**Yield at 30% is 53% based on drug (not including samples purposefully removed)

**Yield at 30% is 66% based on samples/input materials

$$D_n = A_n + B_n - C_n \quad A_{n+1} = C_n - (E_n + F_n + G_n)$$

* Brush down of Wurster unit yields 33 grams

** Includes brushed-down material

TABLE 5

ETIDOCAINE·HCl MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Microcapsules Size Range (μm)	% Drug in Microcapsules		
	90%	80%	70%*
>600	25**	-	1.1
425-600	0.7	-	2.1
300-425	0.4	7.6**	6.4
212-300	3.0	5.2	7.8
150-212	14.2	12.5	10.9
106-150	19.4	20.2	18.8
75-106	31.7	31.6	24.5
38-75	3.2	21.1	27.3
<38	2.3	1.8	1.1

* Does not include brush-down material

** >300 μm , small sample

*** Removed from bed

flowable etidocaine-HCl. The charge of 300 grams of drug was fluidized and coated with 3% polymer solution (original R.S.V. = 1.19 dl/g polymer) at 10 ml/min. Some agglomeration occurred and 73 grams of particles > 500 microns were removed after 9% coating and ground in the Braun mill. This material was returned to the system for further coating. The coating was continued at 10 ml/min. to achieve a 20% and 30% coating, based on the bed weight at the start of each increment of coating. The total coating time was 2.8 hours.

The processing summary is shown in Table 6, and the size distribution in Table 7. There is a wide distribution of size ranges available for testing.

3. In Vitro Drug Release from Microcapsules

Drug release was continued in the L-shaped tubes which were used on the previous contract. The present diffusion bags which hold the microcapsules are formed by folding 38 micron polyester sieve cloth and sewing two sides with polyester thread. The final side is heat sealed with polyethylene after the bag has been filled. Drugs are released into 37°C phosphate buffer at pH 7.4. The system is agitated in a Dubnoff-type shaker. Samples are analyzed by uv spectrometry at 1, 2, 4, 6, and 24 hours as in the previous contract. In the present contract the percent diffusion is based on the drug assay of the particular size of microcapsules being studied, unless otherwise noted.

a. Lidocaine (Base) Release Studies

In the analysis of the drug release from the first Wurster encapsulation of lidocaine (base) it was apparent that the 30% nominal coating was actually very different

TABLE 6

PROCESSING SUMMARY OF BUPIVACAINE-HCl MICROENCAPSULATION (Run 11-3)

Process Polymer %	Starting Weights		Final Weight	Losses				
	Starting Sample g	Polymer Added g		MC (as drug) g (g)	Wurster Holdup g	Oversize Removed g (500 μ m)	Sieve + Bag g	Samples Removed g
0-9	300	30	136 (123)	194	0 ³	21	10 ⁴	
9-20	105	13.8	-	-	-	-	41	
20-30	78	6.6	200 ² (140)	-115	-	-	68 ¹ 132	
TOTALS		50.4		79	0	21	251	
	A	B	C	D	E	F	G	

Material Balance In: 300 g drug + 50.4 g polymer = 350.4 g

Out: 251 g samples + 0 g oversize + 79 g Wurster holdup + 21 g other losses = 351 g

²Yield at 30% is 47% based on drug (not including samples purposefully removed)

³Yield at 30% is 72% based on samples/input materials

$$D_n = A_n + B_n - C_n \quad A_{n+1} = C_n - (E_n + F_n + G_n)$$

¹ Brushed down of Wurster unit yields 132 grams

² Includes brushed-down material

³ 73 g of > 500 μ m ground and returned to bed

⁴ Sample of \approx 250 μ m material

TABLE 7

BUPIVACAINE·HCl MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Microcapsule Size Range (μm)	% Drug in Microcapsules		
	90%***	80%	70%*
>600	-	0.5	0.5
425-600	-	9.4	11.3
300-425	3.0**	18.4	18.1
212-300	12.3	19.5	19.4
150-212	20.4	17.7	15.6
106-150	19.5	15.1	15.8
75-106	19.5	8.8	14.3
38-75	17.1	6.4	4.3
<38	6.8	2.1	2.6

* Does not include brush-down material

** > 300 μm , small sample*** Does not include 73 grams of > 500 μm
which was returned to the bed

for the different size microcapsules. The smallest microcapsules tested (74-105 micron) had only 10% polymer coating. In accordance with this coating level, they released their drug very rapidly (Figure 2). Conversely, the largest microcapsules must contain more than 30% polymer, and they release their drug more slowly.

Since release is measured into a phosphate buffer at pH 7.4 and a similar buffer was used for the previous lidocaine·HCl diffusion medium, the slow rate of drug release from the 292-300 micron and 300-425 micron microcapsules should occur in vivo. The drug release from 6 to 24 hours for each of these samples is most encouraging. It is also encouraging that these size fractions comprise about 50% of the bed material.

b. Etidocaine·HCl Release Studies

The drug release from the etidocaine·HCl microcapsules was relatively similar to the previous encapsulation of this drug. However, the run was stopped after rapidly achieving the 30% coating level. Thus a large, useful sample was obtained. The drug release for 20 and 30% coated microcapsules is shown in Figures 3 and 4. The results show little change of drug coating on size and also little change of release rate with different size microcapsules. Again, the release between 8 and 24 hours is encouraging for the 30% coated microcapsules.

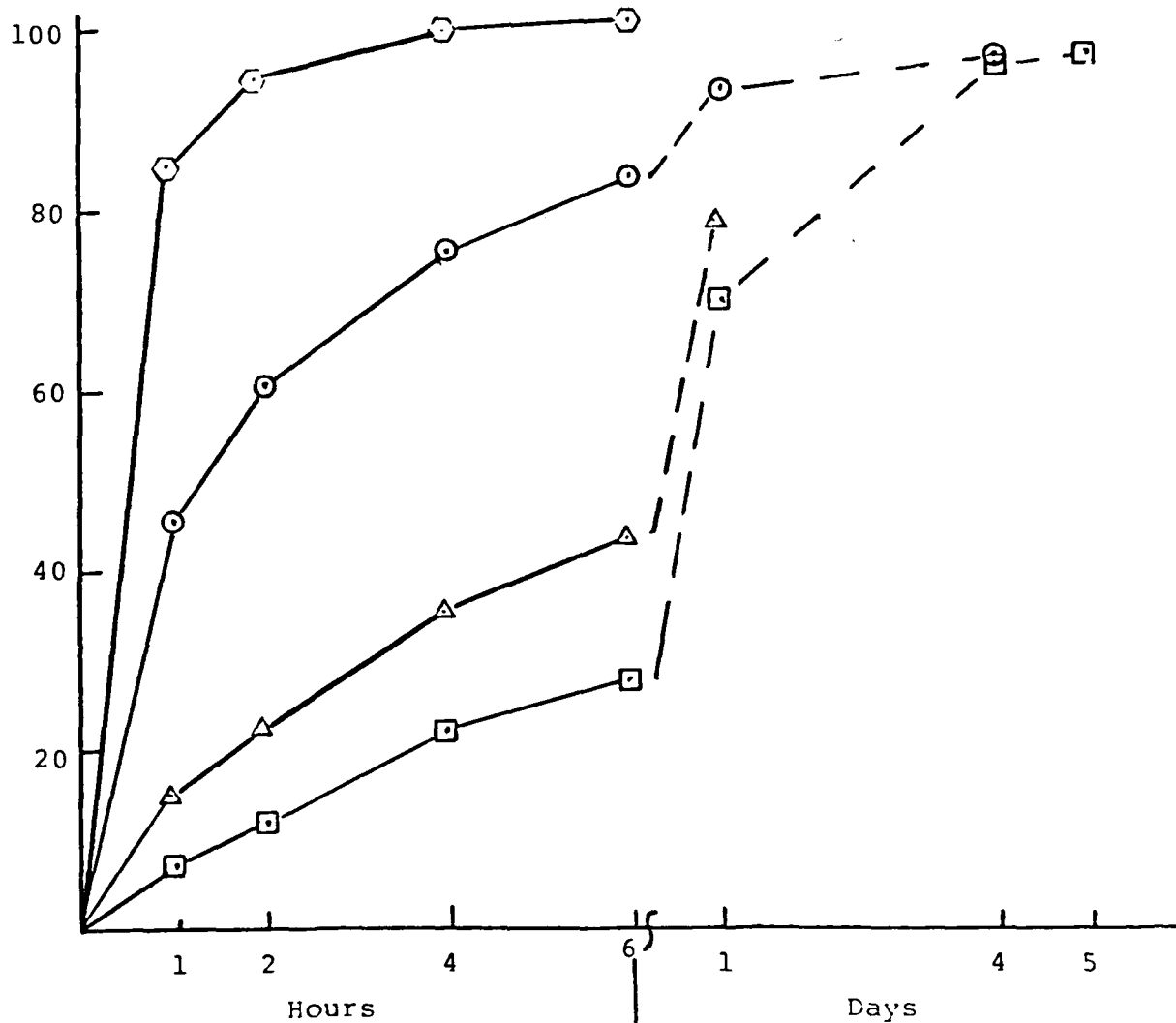
c. Bupivacaine·HCl Release Studies

The release of bupivacaine from microcapsules of the most recent Wurster encapsulation indicates that the smallest particles were poorly coated (Figures 5 and 6, low polymer content and fast release). However, microcapsules of 150 micron and larger are similar in percent coating and in release behavior. Both 20 and 30% nominal coating levels yielded microcapsules having potentially useful drug release profiles.

FIGURE 2
IN VITRO RELEASE OF LIDOCAINE, BASE
FROM MICROCAPSULES (11-1-30)

	Size (μ m)	% Drug	% of Bed
⊗	74-106	90	5
○	150-212	75	8
△	212-300	72	23
□	300-425	68	28

Percent
release



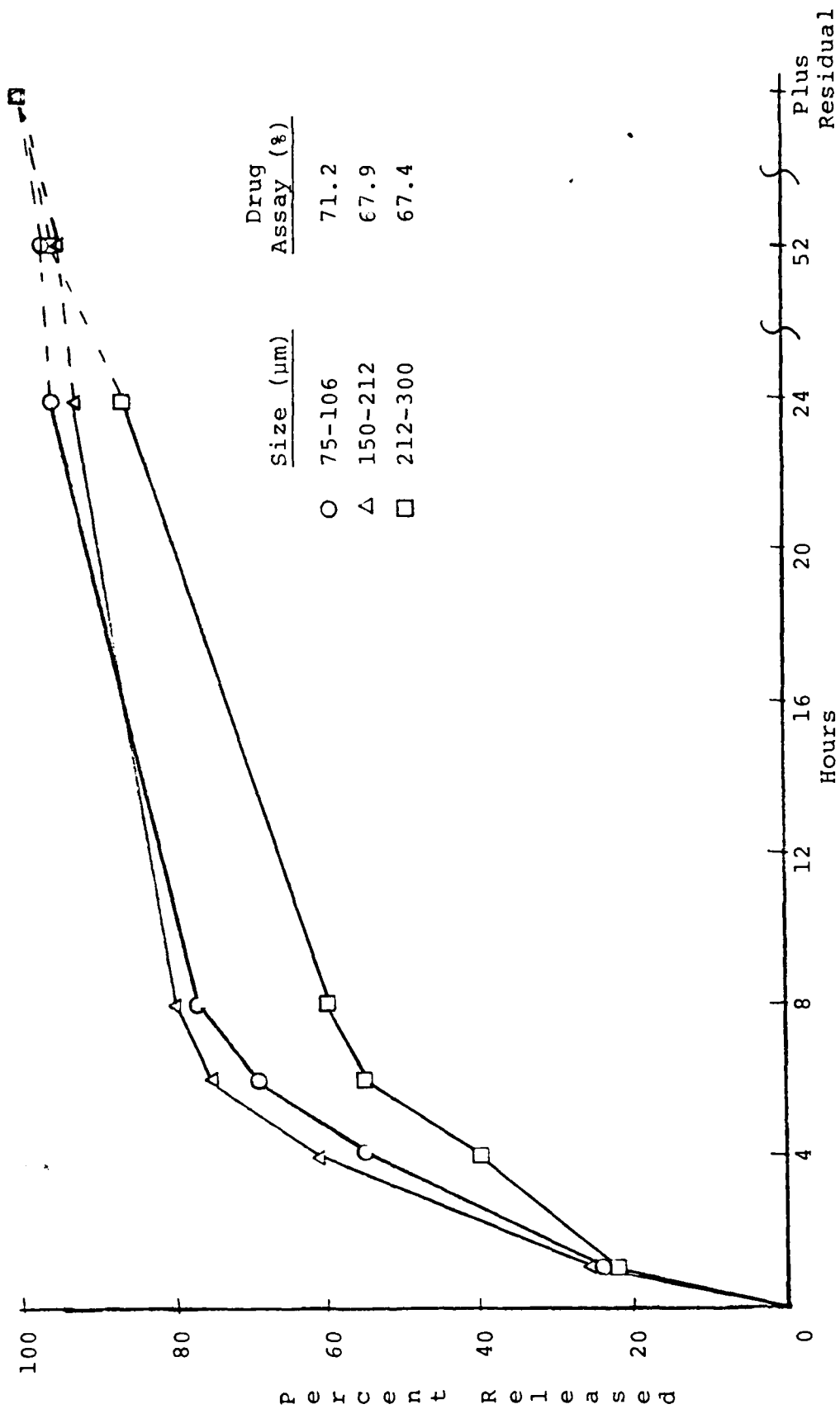


Figure 3 Cumulative Release of Etidocaine·HCl from 11-2-20 Microcapsules
(Corrected to 100% from higher values)

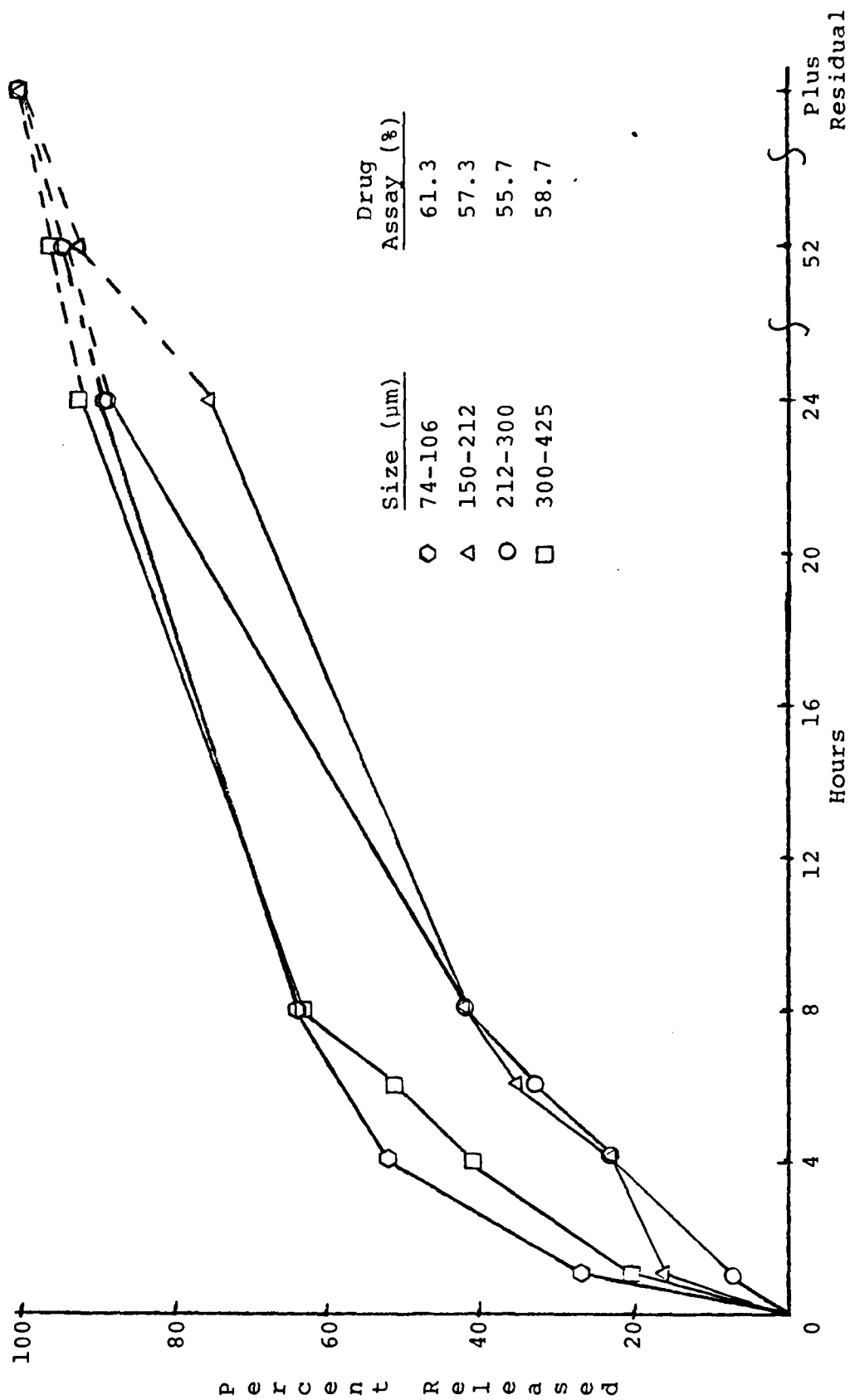


Figure 4 Cumulative Release of Etidocaine-HCl from 11-2-30 Microcapsules
(Values decreased to 100% totals)

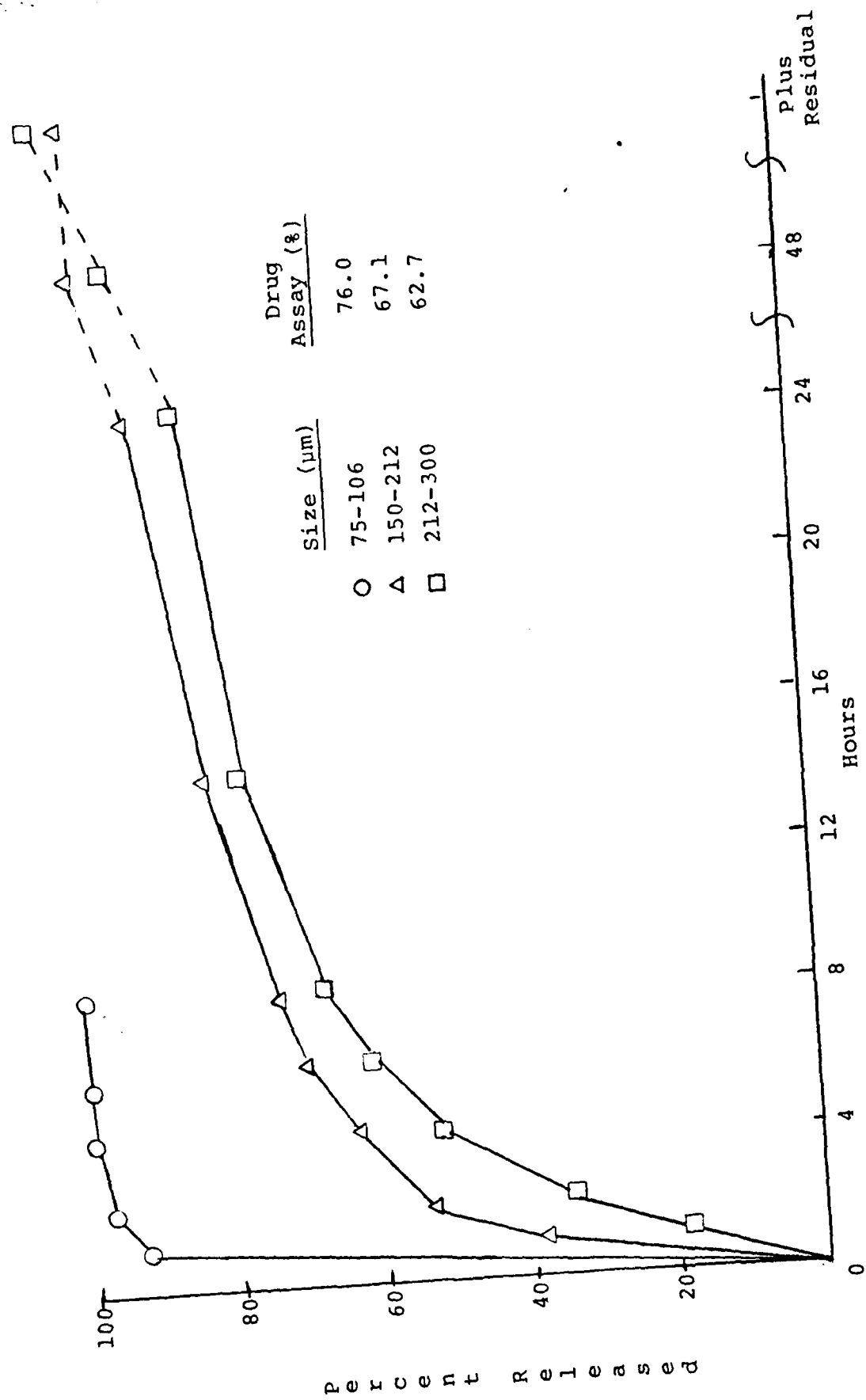


Figure 5 Cumulative Release of Bupivacaine.HCl from 11-3-20 Microcapsules

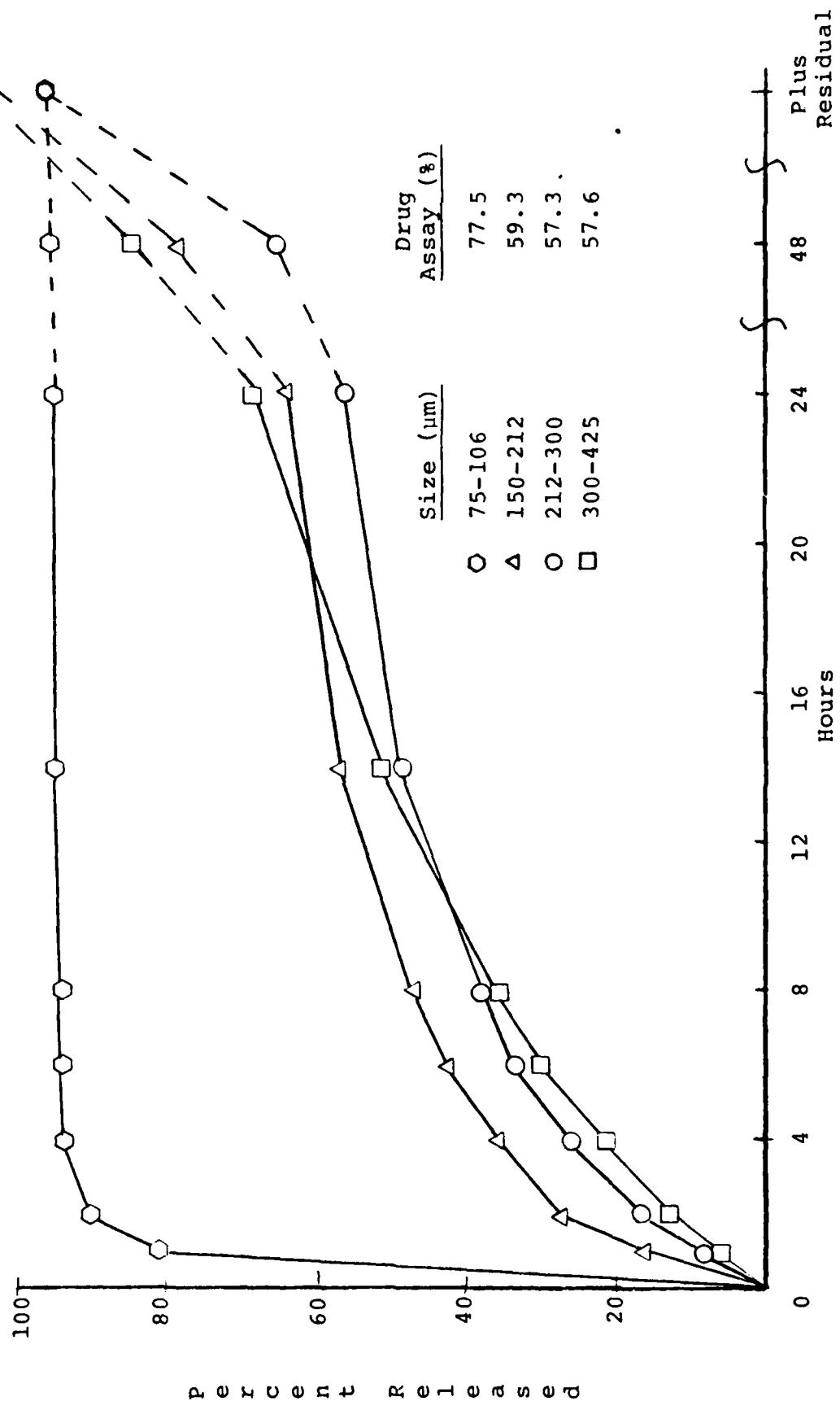


Figure 6 Cumulative Release of Bupivacaine·HCl from 11-3-30 Microcapsules

d. Multi-Point Microcapsule Release Studies

For convenience, data points are normally taken at 1, 2, 4, 6, and 24 hours. However, for the release of drug from selected microcapsules a schedule was devised which allowed more data points to be taken. Separate sets of microcapsules were started in the early morning and late afternoon. Solutions were also measured and changed once in the evening. The data of selected microcapsules are shown in Figures 7 and 8. With this number of points, significant release rate data can also be plotted (Figures 9, 10, 11). These curves are the derivative of the cumulative data which has been plotted previously. The release rate curves show a release equivalent to an infusion process.

The data are generally similar to that obtained previously. The microcapsules varied in age from 2 to 12 months. All the samples were stored in sealed vials at room temperature.

4. Microcapsule Morphology

Scanning electron micrographs (SEM) of microcapsules have been prepared by Colonel Carson Mader of the USAIDR Biophysics Department. Scanning electron micrographs of selected starting materials and microcapsules are shown in Figures 12 and 13.

Because of the time lag in choosing appropriate samples and receiving the photomicrographs, bupivacaine·HCl microcapsules which were prepared at the end of the previous contract are shown in Figure 12. Bupivacaine·HCl (Figure 12.A) is representative of the needle-like crystals of the amide hydrochloride anesthetics. Small crystals are

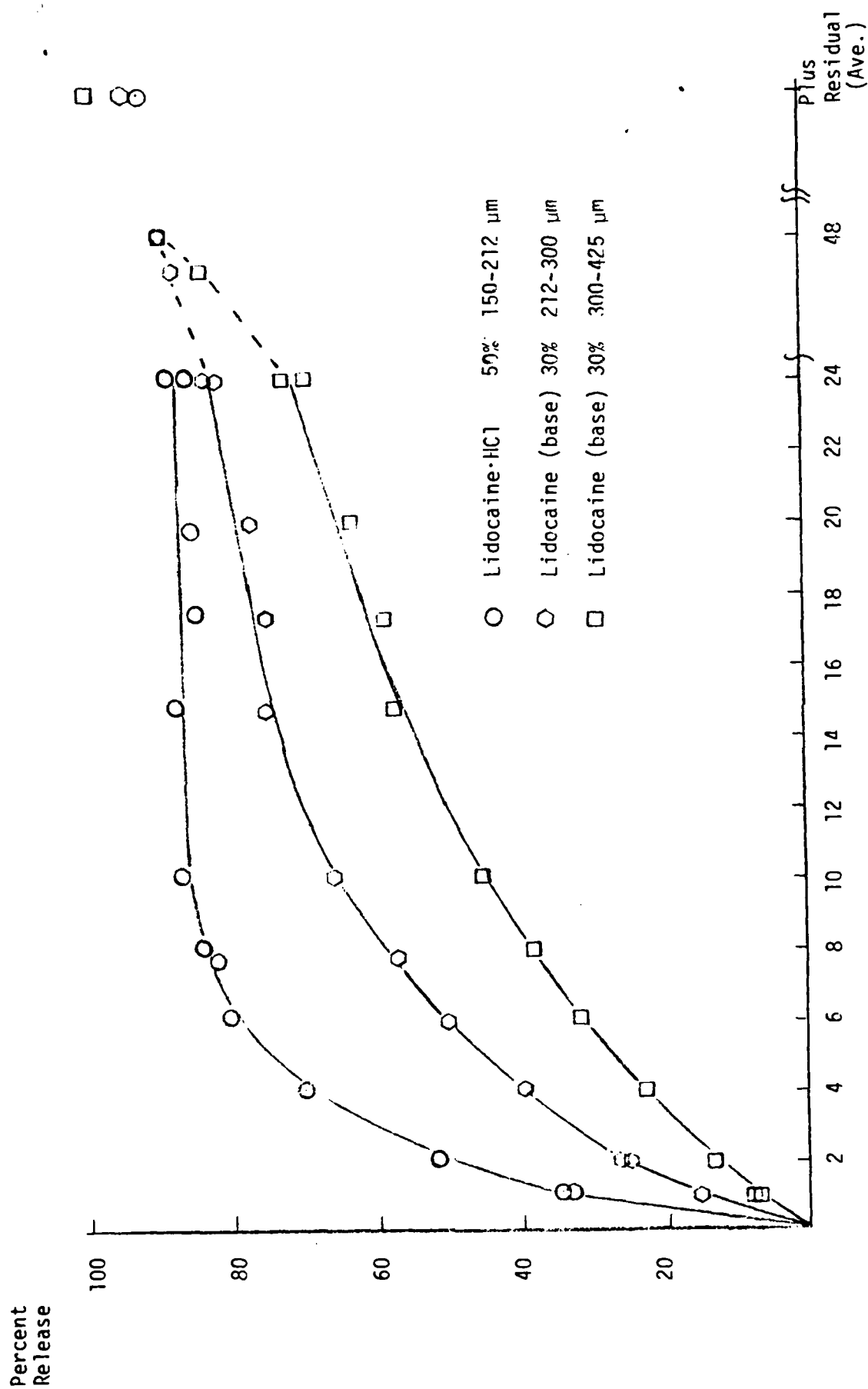


Figure 7 Cumulative Release of Lidocaine from Selected Microcapsules

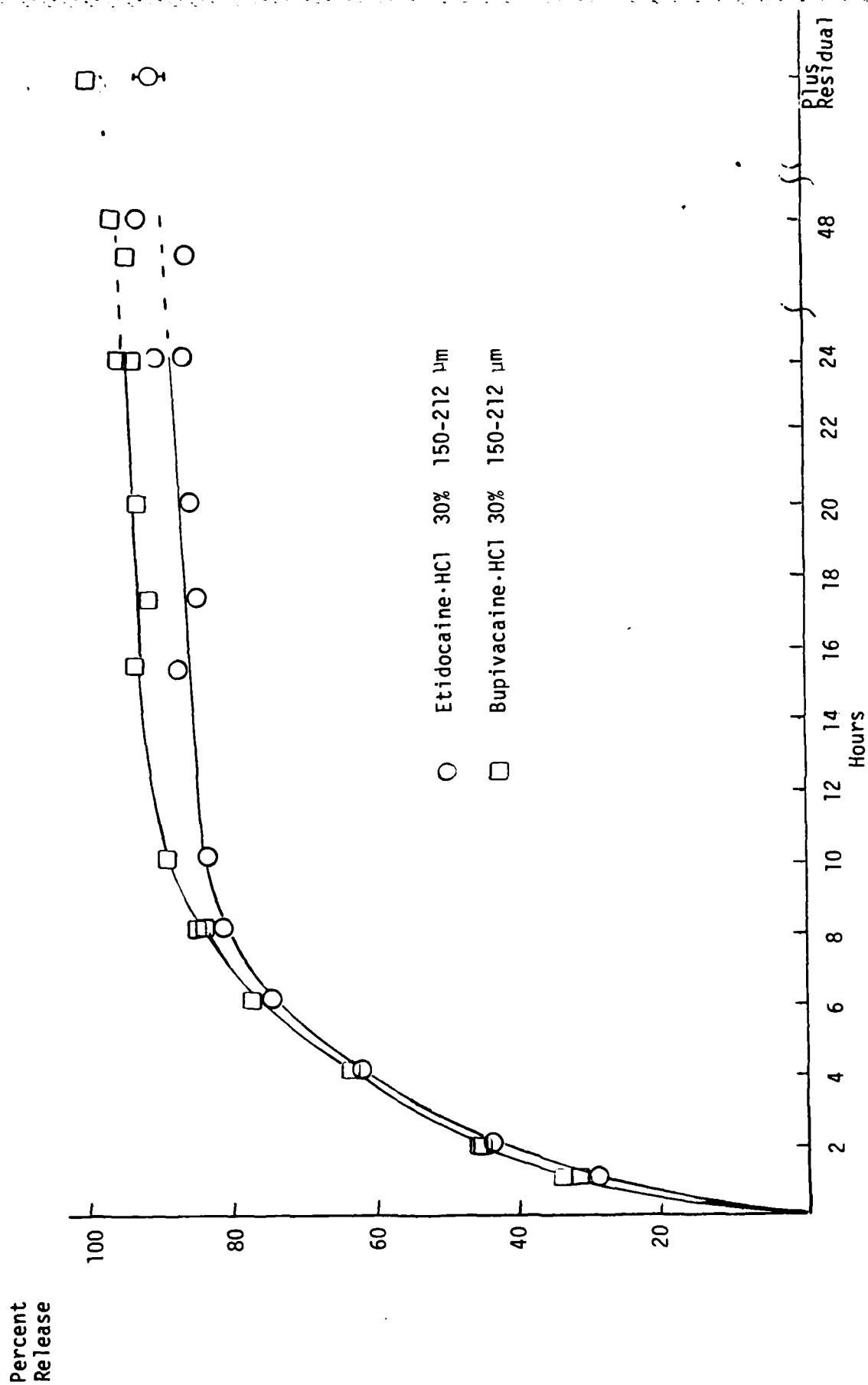


Figure 8 Cumulative Release of Etidocaine and Bupivacaine from Selected Microcapsules

Figure 9 Mean Values of Lidocaine·HCl Release
(μg drug released per mg drug present per hour)

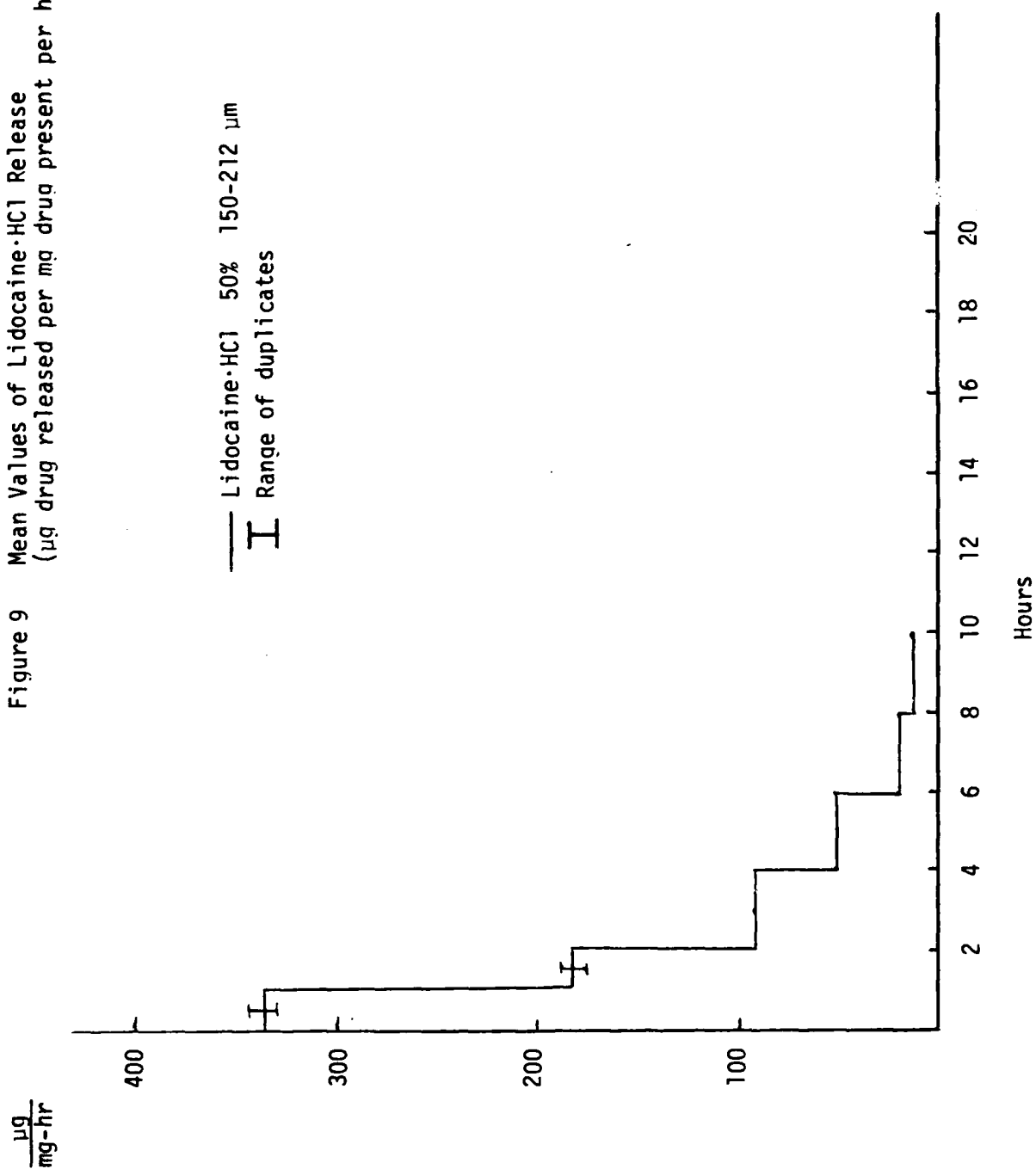


Figure 10 Mean Values of Lidocaine (base) Release
(μg drug released per mg drug present per hour)

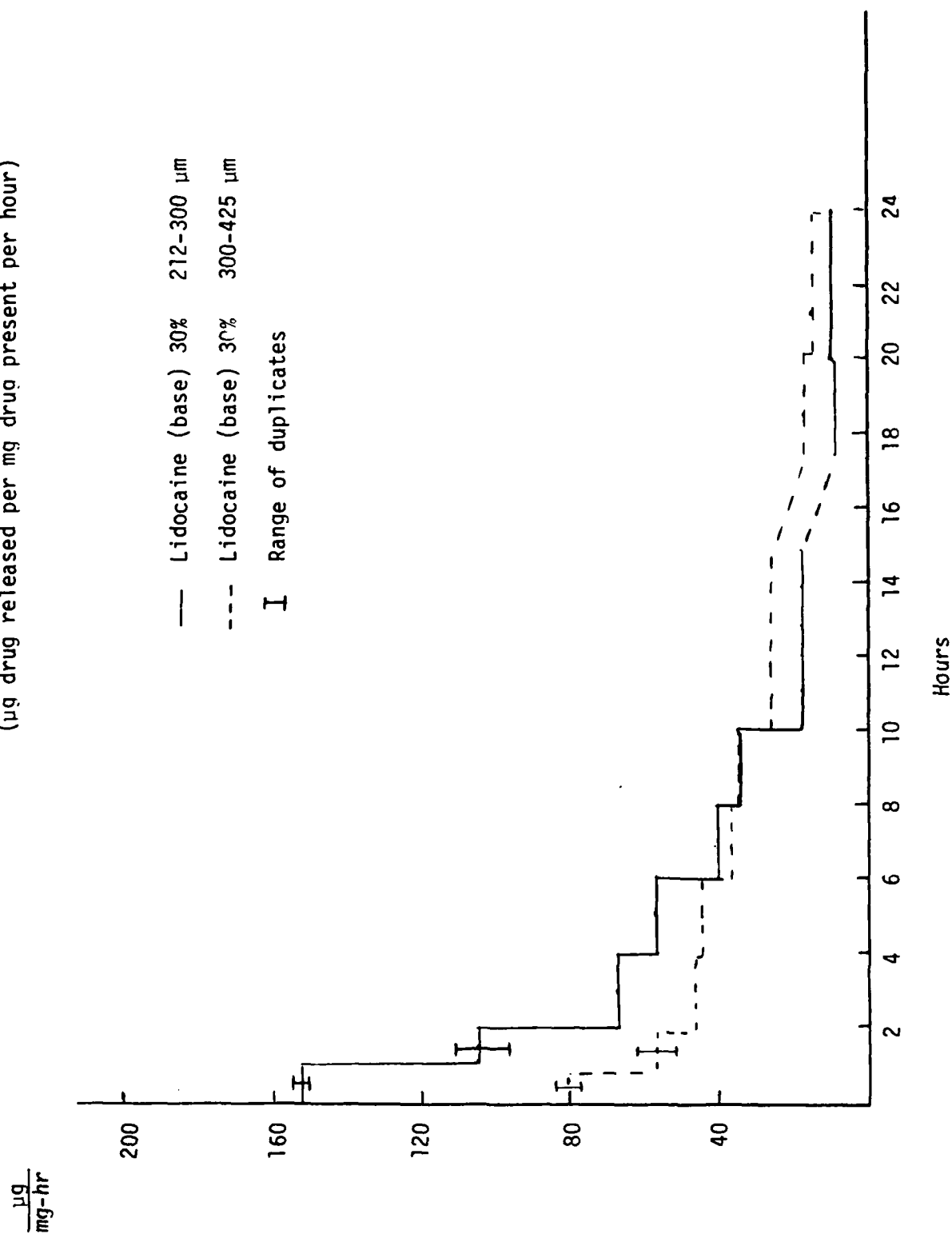


Figure 11 Mean Values of Etidocaine and Bupivacaine Release
(μg drug released per mg drug present per hour)

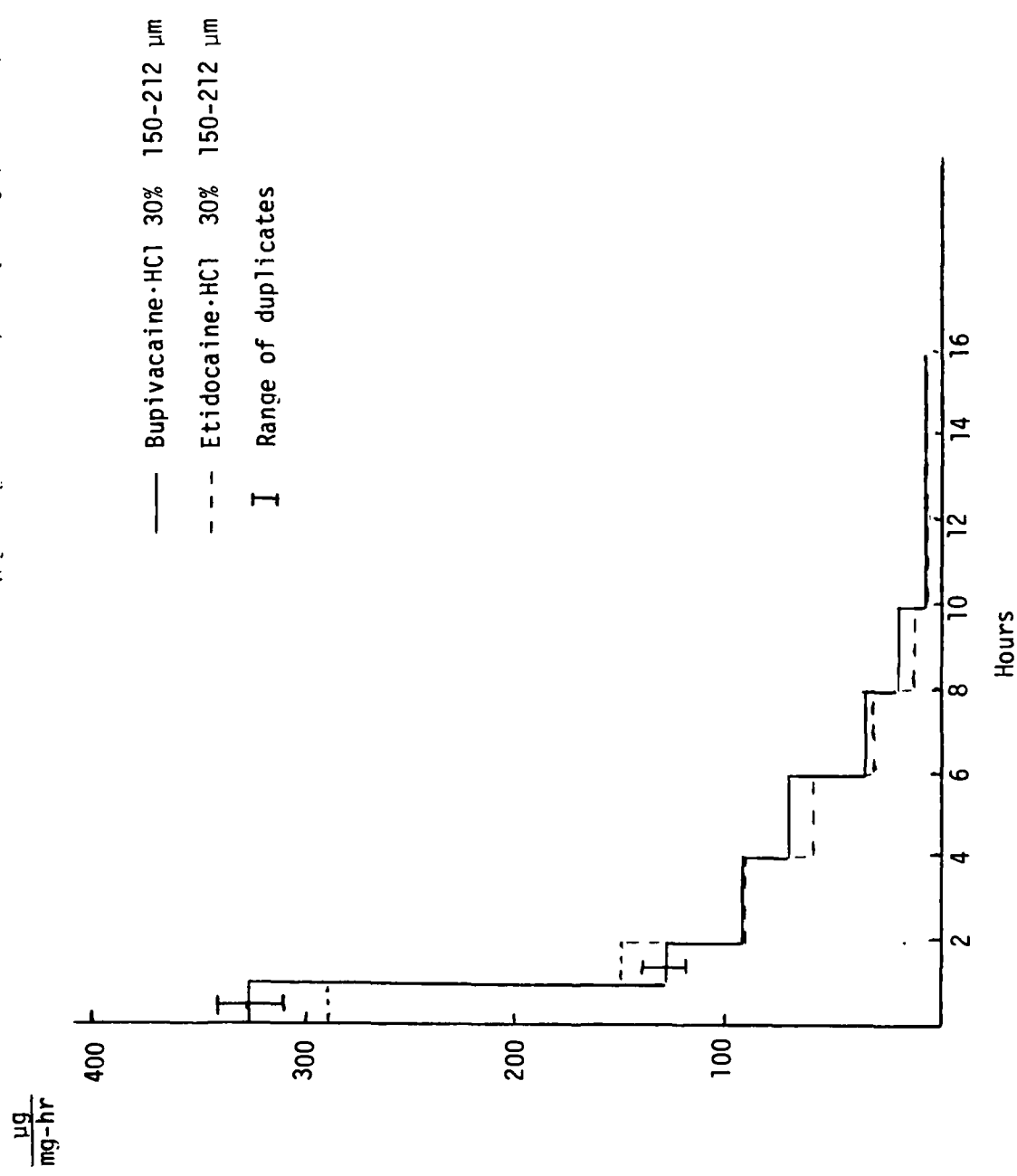




Figure 12
 Group of Experimental Microencapsulation
 Data
 1. Microcapsules
 2. Microcapsules, 75-100 micron microcapsules
 3. Microcapsules, 150-212 micron microcapsules

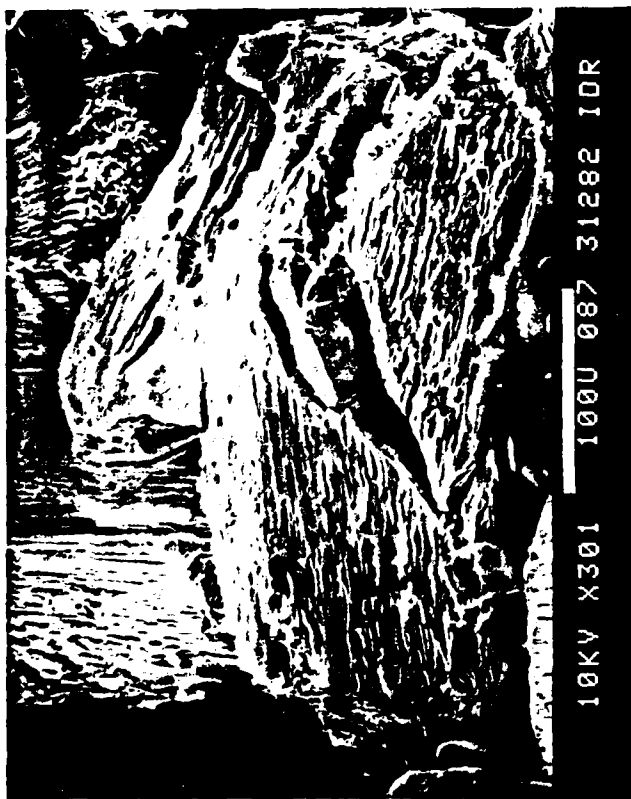
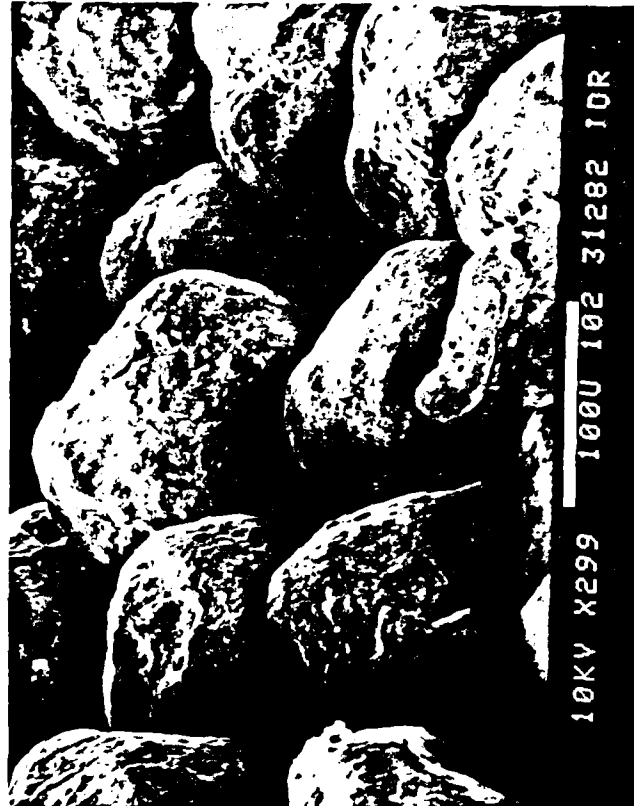


Figure 13
SEMs of Lidocaine (Base) Microencapsulation
(300x)
A) Core Material
B) 30% Coating, 75-106 micron microcapsules
C) 30% Coating, 150-212 micron microcapsules



often imperfectly coated as seen in Figure 12.B.. An amorphous polymer layer normally covers those microcapsules which exhibit useful drug release behavior (Figure 12.C).

Lidocaine (base-USP) is composed of large crystals which apparently break up in the fluidized bed. Agglomerates were also broken up after the bed collapsed during the early stages of coating and again at 13% coating. This process generated a flowable powder which yielded well-coated microcapsules at 30% polymer loading. However, small particles continued to be present which were primarily drug crystals. Many of these particles were covering the walls of the expansion chamber and not being circulated in the fluidized bed.

5. Microcapsule Surface Drug Concentrations

For quality control and correlation to drug release properties, the amount of drug on the surface of the anesthetic microcapsules might also be a useful parameter. In the coating process, a core of drug is covered with polymer. However, because of the direct correlation of drug release and water solubility of various drugs, we have postulated that much of the drug release is probably due to imperfections in the coating. These imperfections could be drug incorporated into the coating.

Fortunately the chloride of the local anesthetic hydrochlorides can be quantitated on the surface by energy dispersive analysis of X-rays (EDAX). A local laboratory performed this analysis with an EDAX 707 and Amray 1000 SEM. Samples were coated with a thin layer of gold-palladium, since graphite coating might cause drug decomposition and loss of hydrogen chloride.

Bupivacaine hydrochloride microcapsules were used as a typical anesthetic microcapsule (70% drug, 150-212 micron sieve fraction). A 20 Kev beam was used. This would penetrate about 2 microns into the sample, assuming a sample density of 1.5g/cm^3 . The radius of the spot on the surface is approximately 1 micron.

Pure bupivacaine hydrochloride had a chlorine peak of 68 c/s (counts/second) under standard conditions. Microcapsules had varying counts/second at this chloride energy peak, depending on the nature of the surface. The normal, smooth, convex surface had a count of 14.7 c/s which calculates to 22% exposed drug. However, flat or concave surfaces, where there is more structure, had counts of 33.3 c/s, corresponding to 45% exposed drug. Most of the surface was smooth.

Thus we have found drug on the surface of the microcapsules. In a uniform appearing surface there are no obvious areas of drug segregation (solid solution or sub-micron domains may exist). In this surface volume there is less drug than expected from a homogeneous particle. Smooth coated surfaces contain less drug than do surfaces with more apparent structure.

6. Microcapsule Stability Studies

Lidocaine and etidocaine microcapsules were stored under six different conditions on April 29, 1981. Selected microcapsules have been studied one year later under the more stringent conditions (40°C, room temperature and light).

There was no significant change of the drug assay or drug release of these microcapsules after one year's storage (Table 8). Bupivacaine microcapsules were stored on July 15, 1981.

Larger quantities of microcapsules described in Table 8 were stored on May 25, 1982. Lidocaine (base) microcapsules (212-300 microns of 11-1-30) were also stored for further study.

7. Selection of Microcapsules for In Vivo Studies

Microcapsules of lidocaine, etidocaine, and bupivacaine hydrochlorides were used in the preliminary in vivo experiments in the preceding contract. However, insufficient quantities of these microcapsules were available for continued in vivo experiments. Based on the in vitro diffusion of various sizes of microcapsules, the choice was made to use the materials in Figure 14. The calculated quantity of available microcapsules is also given in this figure.

TABLE 8
ONE YEAR STORAGE OF SELECTED MICROCAPSULES

<u>Sample</u>	<u>Release Time (Hrs.)</u>	<u>Original</u>	<u>One Year Later</u>		
			<u>40°C No Light</u>	<u>R.T., light</u>	<u>R.T., dark</u>
Lidocaine-HCl	1	36	60	47	41
50% drug	6	81	82	90	78
150-212 μ m	24	83	84	98	83
	Assay %	-	50	44	49
Etidocaine-HCl	1	4	1	3	4
50% drug	6	13	14	13	16
150-212 μ m	24	24	37	32	33
	Assay %	-	46	49	50

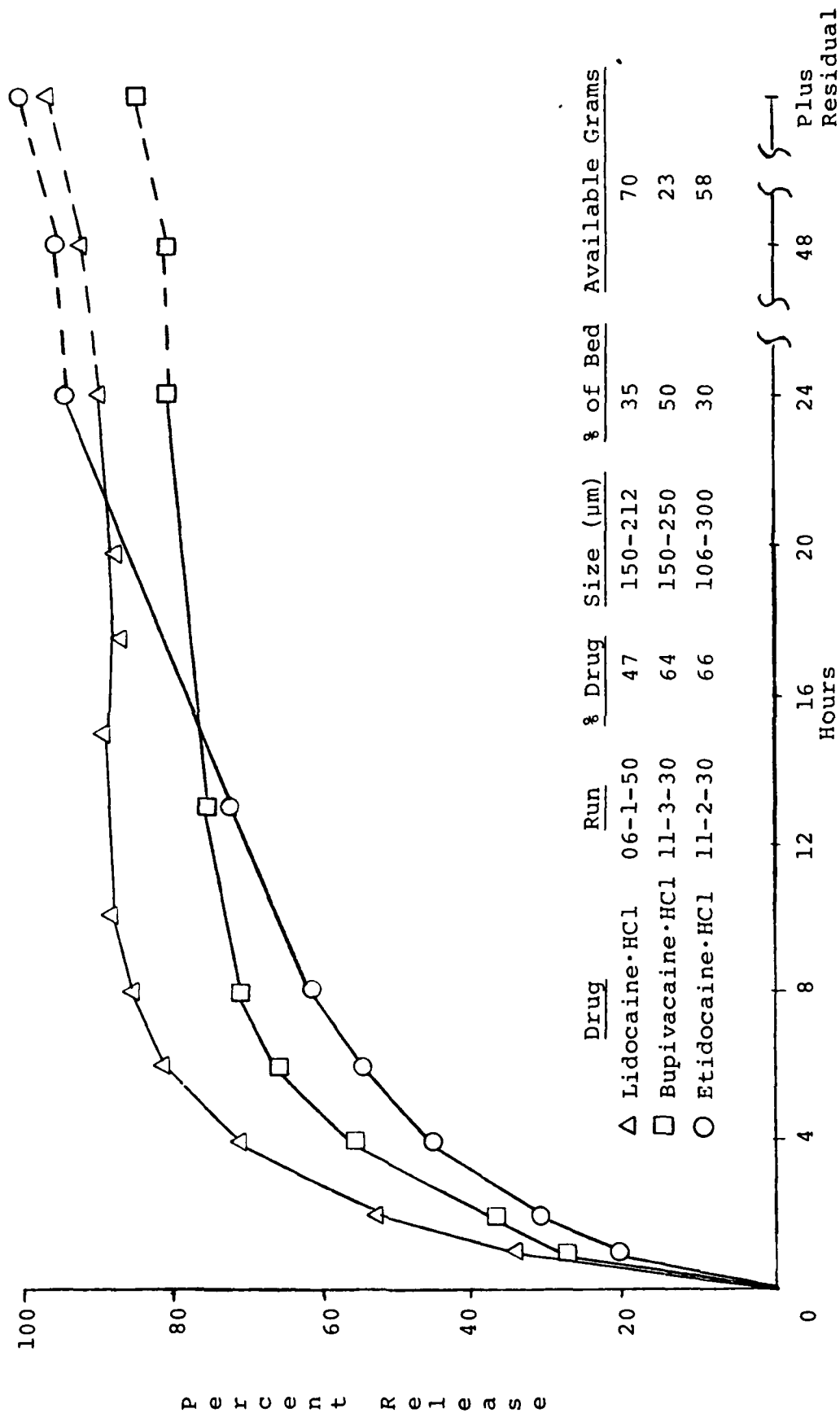


Figure 14 Drug Release and Quantities Available for In Vivo Studies

B. In Vivo Studies

In vivo studies include toxicity testing of the microcapsules relative to the pure drug and anesthetic efficacy testing. Slow release of drug, from the microcapsules, is also to be measured by monitoring blood levels of the anesthetic. Comparison to the blood levels when the pure drug is used in vivo should allow an estimate of in vivo release. This can be compared with the in vitro release for the same microcapsules.

1. Toxicity Testing

Since the systemic toxic effects of the anesthetics are acute reactions (convulsions and death due to respiratory failure) the toxicity may be assumed to be related to the peak concentration of anesthetic in the circulating system. Microencapsulation should decrease this peak value. However, in order to have a longer-acting anesthetic it is also anticipated that more drug may have to be used in the encapsulated form. For acute toxicity testing intraperitoneal injection of the solution and microcapsules was used since a relatively large cavity was required for injection of the microcapsules. This route of administration bypasses the slow absorption of a subcutaneous injection. Oral and intravenous modes are clearly inappropriate.

Toxicity data for lidocaine, bupivacaine, and etidocaine was requested from NIOSH-TIC (Occupational Safety and Health Information System). Over 500 abstracts were received. A recent article by deJong and Bonin (1981) yielded excellent data on the convulsive and lethal doses of all three compounds when injected intraperitoneally in mice. The data are:

<u>Drug</u>	<u>n</u>	<u>CD50</u>	<u>LD50</u>
Lidocaine	48	111.0±6.3	133.1±3.3
Bupivacaine	41	57.7±2.7	58.7±2.0
Etidocaine	60	54.9±2.2	64.4±3.0

John Bonin at Tufts University, Boston, Mass. was contacted about the experimental details. Our major difference is the requirement to use a larger needle in order to inject a suspension of microcapsules. Also a solids suspending vehicle is required to maintain a uniform suspension of microcapsules. We found the suspending vehicle used by NCI to be appropriate. However this 0.3% hydroxypropylcellulose solution in saline is no longer produced by Carter-Glogon Laboratories. It was produced under contract to NCI and six bottles of this material were supplied to us, gratis, by Nathaniel Greenberg at NCI.

a. Preliminary LD50/CD50 Experiment

Following the method of deJong and Bonin (1981) we obtained 10 outbred virgin female white Charles River CD-1 mice. They were ordered as 8-week mice and used at 10 weeks. However, the weights were 24.8±1.9 gm instead of the 30 gm mice used by deJong and Bonin. As expected an 18-gauge needle is less than ideal for intraperitoneal injections. However, it is necessary in order to inject a suspension of microcapsules. By tipping the head back after making the injection, while keeping the needle in the cavity, the solution does not ooze back out as the needle is removed. Finger pressure is also applied over the puncture hole as the needle is removed.

Injection of 0.30 ml of 11.2 mg/ml lidocaine-HCl solution (3.36 mg drug) in hydroxypropylcellulose (HPC, 0.3%) and saline (0.9%) caused convulsions about 4 minutes after the injection. Very shallow breathing followed but the mouse was back on its feet within 20 minutes. This is approximately the LD50 as published by deJong and Bonin.

A suspension of microcapsules was injected into the last three mice. This suspension was successfully injected within 5 minutes of adding the suspending vehicle. However, the suspension was not uniform. The suspension (2 ml) contained 50 mg/ml of 50% drug microcapsules of 150-212 microns. This is a 5% suspension containing 25 mg lidocaine-HCl per milliliter. Injection of 0.42 ml (10.5 mg drug, if uniform suspension) did not cause convulsion or death in the first two mice. The remaining material was rather difficult to suspend and 0.30 ml caused the animal discomfiture, such as dragging of feet.

In the laboratory the remaining drug was measured in the syringes and vial used for the microcapsule suspension. The results are shown in Table 9 and the non-uniformity of the suspension was confirmed.

In order to improve the uniformity of the delivery of microcapsules the vial was changed to a 1 dram bottle which would accept a GC septum cap. This gave fewer available crevices for storage of powder and gave a better view of the suspension. This vial was prepared with 97.6 mg of the same microcapsules, and 2.0 ml of suspending vehicle was added. Five aliquots of 0.3 ml were removed, dried, and weighed. Each aliquot should contain 18.2 mg (14.6 mg of microcapsules and 3.6 mg of NaCl and hydroxypropylcellulose). The weighed containers gave weight increases of 18.9 ± 2.5 mg.

TABLE 9

MATERIAL BALANCE AFTER IP INJECTION
OF LIDOCAINE·HCl MICROCAPSULES

Total drug used	97.6 mg (microcapsules) x 47% drug	45.9 mg
Drug left in vial	17.7	
Drug left in syringe (1) (~ 0.08 ml)	1.3	
(2)	~1.3	
(3)	<u>5.6</u>	
Total not injected		<u>25.9</u> mg
Total injected (by difference)		20.0 mg

Based on these experiments a protocol was written for the injection of 64 mice with four levels of microcapsules and four levels of soluble lidocaine-HCl (8 mice/group with 4 trials at increasing doses and 4 at decreasing doses). The method was readily modified for other drugs, since lidocaine concentrations are higher than those required for the other drugs.

b. Lidocaine-HCl Microcapsule Toxicity

One hundred CD-1 female mice were ordered at 56 days old. Their weights were 26.0 ± 1.4 gm ($n=20$), and therefore no adjustment was made for the differences in weights. Doses were prepared at 30% incremental differences around the CD50 to LD50 doses. The experiment was performed when the mice were 59 days old. From the data of the series of 64 mice, a lower concentration of solution and higher suspension loading was required. These experiments were performed in one day. Solution and suspension concentrations were 112 mg/11 ml and 127 mg/1.7 ml, respectively. Volumes were 0.23, 0.30, 0.39, 0.50 ml. The suspension was used within 10 minutes of the time that the aqueous vehicle was added. Three cages were used below each dose holding cage, to hold injected (normal), convulsed, and dead mice. Mice were also marked according to the dosing group. The lower solution dose was accomplished with 0.18 ml of the standard solution. More concentration suspensions (30 and 69%) were prepared to allow an injection of 0.5 ml to hold the increased dose. The data of this experiment is shown in Table 10.

Each syringe and bottle was labelled and the solids content were combined, dried, and weighed after the experiment. The calculated quantity remaining was 43.2 mg. This weight included the calculated quantity of HPC and NaCl. The weighed quantities were 47.1 ± 6.4 mg. Thus we conclude that most of the microcapsules were uniformly suspended and delivered.

TABLE 10

TOXICITY OF LIDOCAINE·HCl IN SOLUTION AND MICROCAPSULES

	Solution [mg (drug)/kg (mouse)]					Suspension* [mg (drug)/kg (mouse)]				
	68	89	112	150	195	402	523	671	872	1132
Not convulsant	7	5	0	0	0	8	2	0	0	0
Convulsant	1	3	8	7	3	0	6	6	4	1
Dead	0	0	0	1	5	0	2	2	4	7
CD50**	91.6±6.7					488±42				
LD50**	181±12					838±79				
LD50/CD50	1.99					1.72				
M.C. Safety Factor										
CD _{mc} /CD soln.						5.33				
LD _{mc} /LD soln.						4.60				

* Microcapsules of 150-212 micron, 50% lidocaine·HCl, Run 6-1-50

** Calculated by method of Berkson, 1953, following Example 2

We observed similar reactions with both soluble and microencapsulated lidocaine, and with the other anesthetics. Our values for the solution vary from those obtained by deJong and Bonin (1981). They found a lower lethal and higher convulsive dose than we found (Table 11). Our temperature was maintained at 21°C rather than 38°C and our vehicle contained hydroxypropylcellulose and therefore had a higher viscosity than the saline used by deJong and Bonin. Some ambiguity exists between convulsions and a normal righting reflex after a mouse falls over. Because of the nature of these measurements we are not surprised by these differences.

The important factor is the difference in toxicity between the pure and encapsulated drug, when injected intraperitoneally. Based on both convulsive and lethal toxicity end points, the encapsulated drug is approximately five times less toxic than the pure drug. The lower toxicity was anticipated and more drug is expected to be required to produce anesthesia for a prolonged period of time.

c. Etidocaine and Bupivacaine Microcapsule Toxicity

Etidocaine and bupivacaine·HCl microcapsules were compared to the soluble anesthetic using the same general protocol. Preliminary dosing experiments indicated that bupivacaine microcapsules were significantly less toxic.

For etidocaine·HCl, solutions were prepared at 40 mg/10 ml, and suspensions were prepared at 40 mg/2.0 ml of vehicle. These were dispensed in 0.23, 0.30, 0.39, 0.50, 0.50, 0.39, 0.30 and 0.23 ml volumes. For etidocaine·HCl microcapsules, a higher dose was required (80 mg and 3.1 ml vehicle, for 0.50 ml dosing). The data are shown in Table 12. The animal weights for this experiment were 26.4 ± 1.2 grams (57 days old). Again our CD50 was lower than obtained by deJong and Bonin (Table 11).

TABLE 11
COMPARISON OF PRESENT TOXICITY DATA
WITH deJONG AND BONIN (1981)

		<u>Present Study</u>	<u>deJong/Bonin</u>
Lidocaine·HCl	LD50	181±12	133.1±3.3
	CD50	91.6±6.7	111.0±6.3
Etidocaine·HCl	LD50	62.3±6.9	64.4±3.0
	CD50	47.3±4.5	54.9±2.2
Bupivacaine·HCl	LD50	67.6±4.9	58.7±2.0
	CD50	41.5±6.2	57.7±2.7

TABLE 12

TOXICITY OF ETIDOCAINE·HCl IN SOLUTION AND MICROCAPSULES

	Solution (mg/kg)			75.8	Suspension* (mg/kg)				
	34.5	44.8	58.3		114	148	192	250	325
Not Convulsant	7	4	2	0	7	7	3	2	0
Convulsant	1	3	4	0	1	1	3	4	0
Dead	0	1	2	8	0	0	2	2	8
CD50			47.3±4.5				191±21		
LD50			62.3±6.9				260±34		
LD50/CD50			1.32				1.36		
M.C. Safety Factor									
CD50 _{mc} /CD50 soln				4.04					
LD50 _{mc} /LD50 soln				4.17					

* Microcapsules of 106-300 microns, 70% etidocaine·HCl, Run 11-2-30
Drug Assay of 66%

For bupivacaine·HCl microcapsules, the available mice were 64 days old and weighed 28.9 ± 1.2 grams. Bupivacaine·HCl solutions were prepared at 40mg/10 ml of vehicle. Suspensions were prepared at 100 mg of microcapsules in 2.6 ml of vehicle. The high dose was achieved in separate studies using more concentrated solutions and suspensions. Lower toxicity was observed with the bupivacaine·HCl microcapsules (Table 13). This higher "safety factor" of 6.7 is related to the slower drug release of the bupivacaine microcapsules relative to the lidocaine and etidocaine microcapsules. For solutions, we obtained a higher LD50 and lower CD50 than deJong and Bonin (Table 11).

2. Anesthesia Testing

a. Anesthesia Test Materials

After the proposal was submitted, preliminary experiments were performed using a physiograph to give an objective and quantitative value for response to a tactile stimulation. These experiments were described in a letter to Ms. May dated 9 September, 1981. This information led to a contract modification.

Dr. Judson Wynkoop at USAIDR also had developed a tactile stimulator which is based on a spring-loaded syringe needle. This device was kindly sent to us for reproduction. Appropriate engineering sketches were drawn, and the device was fabricated by a local machine shop. After a few problems with the device, a roll pin was placed into the cylinder to prevent the knurled stop from being lowered to a point at which the needle hub hits the end plate. A 15-gauge needle was cut and rebevelled, such that it protrudes 5 mm from the end plate.

b. Preliminary Anesthesia Experiments

Our consultant, Dr. Michael Gay, is attempting to develop a method to test the efficacy of local anesthetics, based on his earlier experiments which were described in our request for additional funds. The Narco Physiograph was

TABLE 13

TOXICITY OF BUPIVACAINE·HCl IN SOLUTION AND MICROCAPSULES

	Solution (mg/kg)					Suspension* (mg/kg)				
	31.9	41.3	53.9	70.1	91.1	184	239	311	404	525
Not Convulsant	8	4	1	0	0	8	6	1	2	1
Convulsant	0	4	7	3	0	0	2	7	4	1
Dead	0	0	0	5	8	0	0	0	2	6
CD50			41.5±6.2					280±44		
LD50			67.6±4.9					453±40		
LD50/CD50			1.6					1.6		
M.C. Safety Factor										
CD50 _{mc} /CD50 _{soln}					6.7					
LD50 _{mc} /LD50 _{soln}					6.7					

* Microcapsules of 150-250 micron of 70% bupivacaine·HCl, Run 11-3-30
Drug Assay of 60%

received and was used at BIOTEK by Dr. Gay and his graduate student, Demetris Hadjilambris. They are also using the USAIDR tactile stimulator. The specific objective of these experiments is to develop a simple, reliable method for observing responses to a painful stimulus.

Rabbits were chosen primarily because blood levels of the local anesthetic agent were to be determined during potency and duration testing. Needle electrodes were placed subcutaneously 2 cm apart along the midline of the back, near the sacral-lumbar junction. The direct and integrated electromyograph (EMG) was recorded from these electrodes using a Narcobiosystems Physiograph with a high gain preamplifier and an envelope type, full-wave rectifying integrator. Respiration was recorded from two needle electrodes placed subcutaneously on opposite lateral chest walls using the Narco impedance pneumograph. Local anesthetic agents were administered either subcutaneously or intradermally on the upper thigh and lower back.

1) Intradermal Rabbit Studies

For intradermal tests, four doses and vehicle control were tested simultaneously in separate wheals. The tactile stimulus was a needle prick delivered by the USAIDR device. When the observer delivered the stimulus, the event pen of the recorder was activated. If a twitch response was observed, the remote switch for the event marker was immediately released. If no response occurred, the event marker was not released for at least 3 seconds. Thus the observed twitch response, EMG, and respiration were simultaneously recorded. Later the event marker was automatically

tripped by the travel of the barrel of the USAIDR stimulator. It was also found necessary to build a rabbit restrainer which allowed access to the hind quarter of the rabbit, but minimized random movement of the rabbit.

Each test trial consisted of five stimuli to each injection site and five trials were performed over a 30-minute period. Data were recorded as either number of negative responses (local anesthesia) or percentage of the 25 possible responses that were negative. If a stimulus resulted in an immediate, integrated EMG response of 2 mm or more, as determined by vertical relationship to the event marker, it was defined as positive. The height of the response in mm was also measured.

The sample records show that the integrated EMG has a stable baseline, and response to the stimulus is easily detected. This is superior to the direct EMG response. The respiratory response to stimulation is a short gasp which is the most sensitive measure. However, since the baseline recording is very unstable, this response was not used.

There was a major problem with this method. Five rabbits were tested on two sessions at least 7 days apart, with one trial on each side during each session. Following injection of 0.2 ml of saline vehicle intradermally, $17.2\% \pm 4.6$ of the observed twitch responses were negative. When lidocaine·HCl was being tested the control sites gave $27.8\% \pm 2.3$ negatives, and when procaine·HCl was being tested $7.6\% \pm 4.4$ control negatives were observed. There

does not appear to be any pattern in the occurrence of these false negatives. They occur equally in the EMG recordings and in the observed twitch response. An unacceptable level of variability is also seen in mm of the EMG response. The standard errors are 32% and 11% of the means for saline controls in the lidocaine and procaine tests, respectively. For the remaining analysis (Tables 14 and 15) all animals with greater than 20% control negatives were eliminated.

A second problem is that the log dose response curves are not linear. As shown in the tabulated data, the highest correlation coefficient for log-linear regression was 0.88. The animal-to-animal variability is so great that any dose relationship is obscured. In most cases there is no significant difference between doses. This is probably not a result of incorrect dose range, since the means of the observed twitch responses were between 25% and 75% negative responses.

The final problem is that even at the highest doses, about 25% of the time the animal still responds to the tactile stimulus.

The method follows that of Bulbring and Wajda (1945) in which they injected 0.0125% to 0.1% of nupercaine intradermally in guinea pigs. They obtain 100% response to a tactile stimulus after the anesthetic wears off. They also have 0% response for the first 20 minutes when 0.1% anesthetic was used. Data is also shown for procaine (0.25 to 2.0% drug) with a similar general effect. For lidocaine, Camougis and Takman (1971) report complete anesthesia (12 of 12) for 70-90 minutes with 0.1 ml of 2% drug. A 0.5% solution decreased the duration to about 30 minutes. However, all of these experiments used the classical guinea pig animal model.

TABLE 14
INTRADERMAL DOSE RESPONSE DATA
USING 0.2 ml OF PROCAINE-HCl IN SALINE
IN RABBITS

Drug Concentration %	Observation Twitch % Negative	Integrated EMG Data		
		% Negative	mm	mm-Control
0	3.5±1.8*	4.4±2.0*	619±70*	-
0.25	44±10	36±9	228±57	391
0.50	61±10	52±8	141±64	478
1.00	70±9	50±8	163±50	456
2.00	68±8	48±12	170±55	449
**r	0.88	0.61	-0.52	0.53

* Control is significantly different from each concentration of drug ($p < .05$).

** logarithmic dose relationship; does not include control (0%).

TABLE 15
INTRADERMAL DOSE RESPONSE DATA
USING 0.2 ml OF LIDOCAINE·HCl IN SALINE
IN RABBITS

Drug Concentration %	Observed Twitch % Negative	Integrated EMG Data		
		% Negative	mm	mm-Control
0	8.0±4.9*	8.0±3.4*	391±124	-
0.125	69±10	51±9	176±49	215±88
0.25	56±4	40±6	193±53	197±94
0.50	84±6	59±10	152±60	238±90
1.00	74±8	62±9	154±48	236±96
r	0.47	0.68	-0.71	0.77

* Control is significantly different from each concentration of drug ($p < .05$)

In our experiments the EMG recordings gave very similar results to the observed movement. However, this is not very valuable. It seems most reasonable to compare the EMG method to the observed twitch response method using the classical model of the guinea pig. If this experiment, with EMG and USAIDR stimulator, gives data comparable to that of Bülbbring and Wajda, then the rabbit is an inappropriate model for intradermal injection of local anesthetics. If the data are not improved, when the guinea pig is used, then a problem may exist with the stimulator or EMG signal pick-up and recording methodology.

2) Subcutaneous Rabbit Studies

The interdermal wheal is the standard method of determining anesthesia of nerve endings on the skin surface. However, microcapsules cannot be injected intradermally (25 gauge needle) and a subcutaneous route was therefore proposed. A subcutaneous injection pilot study was performed for testing duration and extent of local anesthesia in the rabbit. Table 16 shows the duration of action of several volumes of 1.0% lidocaine-HCl in saline injected subcutaneously. These data are the observed twitch response following stimulation at the injection site.

In this experiment, anesthesia spread 1.0 cm from the injection site following an 0.2 ml injection volume, and 4.0 cm following an 0.4 ml injection. This 4.0 cm radius is nearly equal to the total area of the rabbit's hind quarters.

The results of this experiment are encouraging. However, since the same nerve sensors and apparatus are involved, we must question the validity of this approach.

TABLE 16
SUBCUTANEOUS INJECTION OF LIDOCAINE·HCl
IN RABBITS

<u>Method of Injection</u>	<u>Total Drug (mg)</u>	<u>Duration of Action (minutes)</u>	<u>Radius of Action (cm, max)</u>
Solution in saline			
(0.2 ml, 1%)	2	29	1.0
(0.4 ml, 1%)	4	69	4.0
(0.5 ml, 1%)	5	73	-
(0.75 ml, 1%)	7.5	81	-
Solution in HPC			
(0.4 ml, 1%)	4	75	1.5
Capsule Suspension in HPC			
(0.4 ml, 10% x 50% drug)	20	75	1.8
Trocar with dry crystals			
(20 mg)	20	40	2.5
Trocar with capsules			
(40 mg x 50% drug)	20	40	1.5

Microcapsules can be injected subcutaneously, as a suspension in HPC, if a large needle is used (16-18 gauge). A very large needle (e.g. 12 gauge) can be packed with dry powder which is pushed out with a rod, as in a trocar. Preliminary data are shown in Table 16. In addition to the general problem of surface anesthesia in the rabbit, the microcapsules were observed to fall several centimeters from the point at which they were injected by trocar. The solid microcapsules could be felt under the skin. The dorsal surface of a rabbit (or guinea pig) may be a tighter surface which will allow less movement of a subcutaneously injected drug.

3) Intradermal Guinea Pig Studies

Retired breeder guinea pigs were used by our consultant, Dr. Michael Gay at Northeastern University. A Centrap (Fisher Scientific/Biodec, Inc.) was used as the restrainer. The needle for the USAIDR device was changed from a 15 to 26 gauge needle. The 15 gauge needle broke the skin of the guinea pig, causing unwanted bleeding. The injection volume was increased from 0.20 ml to 0.25 ml, to follow the method of Bülbring and Wajda more exactly. Finally, five of the six guinea pig trials using lidocaine were done in a blind manner, by injection of coded samples.

The guinea pig demonstrates an obvious twitch which is repeatable with even a small mechanical insult. With the rabbit, a gross insult was required and the mechanical skin movement had to be differentiated from a muscle twitch. With the rabbit there was no significant anesthetic logarithmic dose response (\bar{r} range of 0.47 to 0.88). With the guinea pig there is an excellent logarithmic dose response ($r = 0.962$ to 0.9975 , Tables 17-18. With this type of data we can also demonstrate the increased potency of lidocaine versus procaine, and the increased sensitivity of the EMG response versus the visual twitch response (Figure 15, less negative responses with EMG than with visual observation).

TABLE 17
INTRADERMAL DOSE RESPONSE DATA
USING 0.25 ml OF PROCAINE·HCl IN SALINE
IN GUINEA PIGS

Drug Concentration %	Observation Twitch % Negative	Integrated EMG	
		% Negative	mm÷Control mm (%)
0	2.1±1.3	3.1±1.5	-
0.25	36.9±7.2	35.6±5.0	66.5±2.2
0.50	53.6±7.8	42.2±2.2	55.8±17.5
1.00	57.8±7.5	46.7±4.7	29.7±4.0
2.00	74.4±6.1	62.2±7.5	24.7±6.0
* r	0.978	0.962	-0.969

* correlation coefficient, r , is based on logarithm of doses
 (0.25 to 2.00%)

TABLE 18
INTRADERMAL DOSE RESPONSE DATA
USING 0.25 ml OF LIDOCAINE-HCl IN SALINE
IN GUINEA PIGS

Drug Concentration %	Observation Twitch % Negative	Integrated EMG	
		% Negative	mm-Control mm (%)
0	3.2±0.9	2.9±0.8	-
0.125	53.3±11.9	40.6±8.3	38.0±8.1
0.25	77.2±7.5	49.4±6.7	23.0±6.6
0.50	85.6±5.0	62.2±7.5	15.9±3.7
1.00	83.3±3.1	71.6±2.8	11.2±2.3
r	*	0.9975	-0.966

* too few positive responses for statistical analysis

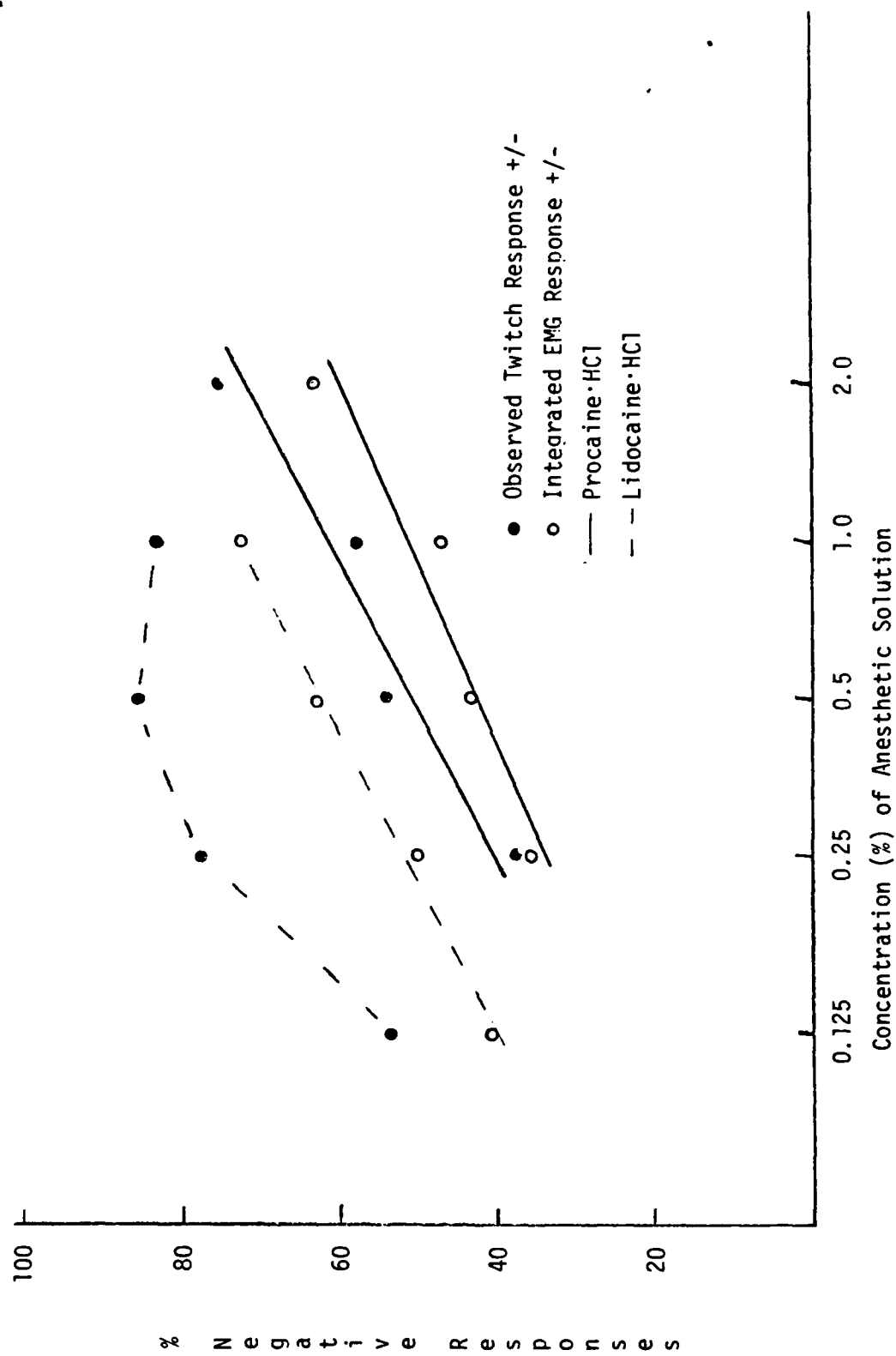


Figure 15 Intradermal Injection of 0.25 ml of Anesthetic into Guinea Pig

Comparison of these results (Tables 17 and 18) with the rabbit data (Tables 14 and 15) indicate that the animal model, not the equipment, was the problem in repeating the data of Bülbring and Wajda (1945).

In general we believe the guinea pig is the more appropriate animal model because the panniculus carnosus is better developed in the guinea pig than in the rabbit (Donald Smith, Northeastern University, Director Laboratory Medicine, personal communication, Cooper and Schiller, 1975). This is a thin layer of muscular tissue just beneath or within the superficial fascia, which would respond to a cutaneous insult.

Although the guinea pig can be bled with relative ease through the orbital plexus, we cannot obtain sufficient quantities of blood at the necessary time intervals for measurement of anesthetic and metabolite circulating levels by the USAIDR method. Thus the use of the rabbit may be required as a parallel animal model for these experiments.

4) Rat Sciatic Nerve Block

In an independent experiment, etidocaine·HCl and etidocaine·HCl microcapsules were injected into rats to achieve a block of the sciatic nerve. The end point of these experiments is an obvious opening of the toes on the affected leg. This experimental block of a major, sheathed nerve is a classical local anesthetic model. Although wounds may be better treated by surface anesthesia of exposed nerve endings, this experiment

does lead to an anesthetic duration value. The results of this experiment are shown in Table 19. An 18 gauge needle was used to inject 0.25 ml of 1% etidocaine-HCl as a solution or suspension into the tissue area near the sciatic nerve.

It should be noted that several years ago this type of experiment was attempted at Abcor with less success (Contract DAMD17-79-C-9019). However, that experiment was performed with a weaker anesthetic (lidocaine-HCl) in microcapsules with faster release, with a lower percent drug (50%), and through a smaller needle (20 gauge).

TABLE 19

DURATION OF RAT SCIATIC NERVE BLOCK

FROM SOLUTION AND SUSPENSION OF

ETIDOCAINE·HCl

<u>Rat No.</u>	<u>Anesthetic Duration (minutes)</u>	
	<u>1% Solution</u>	<u>1% as Suspension*</u>
1	68.4	138.5
2	49.1	255.5
3	73.7	103.3
4	52.1	200.1
5	111.5	178.0
Ave.	71.0	175.1
S.D.	25.0	58.3
S.E.M.	11.2	26.1
t	3.67	
p	<0.01	

* Run 11-2-30, 106-300 micron microcapsules

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